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CYP2C9 Polymorphism and Unstable Anticoagulation with Warfarin in Patients Within the First 3 Months Following Heart Valve Replacement*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Warfarin dose requirements are partly determined by common single nucleotide polymorphisms in *VKORC1* and *CYP2C9* genes.
Objectives. The aim of this study was to investigate how the presence of allelic variants in *CYP2C9* affects the stability of anticoagulation in patients within the first 3 months following elective heart valve replacement.
Material and Methods. In a case-control study we compared 18 consecutive carriers of *CYP2C9*2* and/or **3* and 25 well-matched patients with the wild type *CYP2C9*1/*1* genotype. The former group was randomly assigned to use coagulometers or monitor international normalized ratio (INR) in local outpatient clinics. Subjects receiving drugs potently interfering with warfarin were ineligible. Anticoagulation with the baseline warfarin regimens based on pharmacogenetic algorithm was assessed by time in the therapeutic INR range (TTR) within the first 3 months following implantation.
Results. Carriers of the *CYP2C9*2* and/or **3* genotypes were characterized by lower estimated warfarin dose (median, 21 [interquartile range, 21–35] vs. 35 [28–42] mg/week, $p = 0.02$) and actual (27.8 ± 13.2 vs. 46.3 ± 13.9 mg/week, $p < 0.001$), together with lower TTR values (56 [38.6–74.9] vs. 75.4 [58.1–83.6] %, $p = 0.03$) and longer time above the therapeutic range (13.8 [4.9–34.5] vs. 4.5 [0–15.3] %, $p = 0.047$) than patients with the *CYP2C9*1/*1* genotype. There were no differences in the estimated and actual warfarin doses, TTR values and adverse events between the self-testing and standard-care subgroups.
Conclusions. The presence of *CYP2C9*2* and/or **3* genotypes is associated with unstable warfarin treatment in patients after heart valve replacement, regardless of the type of INR testing (**Adv Clin Exp Med 2015, 24, 4, 607–614**).

Key words: warfarin, TTR, pharmacogenetics, heart valve replacement.

Despite increasing clinical use of direct thrombin and factor (F) Xa inhibitors, mainly in nonvalvular atrial fibrillation (AF), 4-hydroxycoumarin derivatives – vitamin K antagonists (VKAs) – are used in the prevention of valve thrombosis and arterial thromboembolism in patients with artificial heart valves [1–3]. The most commonly used VKA worldwide, warfarin, exhibits its anticoagulant effect by inhibiting the recycling of oxidized vitamin K to the reduced form (KH_2) by vitamin K epoxide reductase complex subunit 1 (*VKORC1*) in the liver. Vitamin KH_2 serves as a cofactor in carboxylation of glutamate residues (Gla) on the N-terminal regions of vitamin K-dependent blood coagulation factors (F), i.e. FII, FVII, FIX, and FX [4]. Treatment with warfarin

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results in the hepatic production of partially carboxylated and decarboxylated proteins with reduced coagulant activity.

However, VKA therapy is challenging because of its narrow therapeutic index combined with the wide inter-individual dosing variation. The efficacy and safety of warfarin treatment are highly dependent on the time in which the international normalized ratio (INR) is in the therapeutic range (TTR) [5]. A maintenance dose of VKA depends on several factors, predominantly diet and medications, or individual features such as age and body weight as well as genetics factors [6]. *VKORC1* and cytochrome P450 (*CYP*) *2C9* genetic variants contribute largely to inter-individual variations in warfarin dose requirements affecting its pharmacodynamics and pharmacokinetics, respectively [7, 8]. The *CYP2C9* gene is located on chromosome 10q24 and encodes the cytochrome P450 enzyme that metabolizes the *S*-warfarin isoform, which is 2.7 to 3.8 times more potent than the R enantiomer [6].

The major *CYP2C9* polymorphisms, *CYP2C9*2* in exon 3 (p.Arg144Cys, c.430C > T, rs1799853) and *CYP2C9*3* in exon 7 (p.Ile359Leu, c.1075A > C, rs1057910) are associated with reduced enzyme activity [2, 6]. *In vitro* study has shown that intrinsic clearance of S-warfarin was 5.5-fold and 27-fold lower when the reaction was catalyzed by enzymes encoded by *CYP2C9*2* and *CYP2C9*3* allelic variants, respectively, compared with the wild type genotype *CYP2C9*1/*1* [9]. Patients with the *VKORC1* wild type and the *CYP2C9*1/*2* genotypes require approximately the same dose of warfarin [10]. In the presence of any of the genotypes – *CYP2C9*2/*2*, *CYP2C9*2/*3* or *CYP2C9*1/*3* together with the *VKORC1* wild type variant, a warfarin dose is decreased approximately 1.7-fold compared to the *CYP2C9*1/*1* variant. Subjects with the *CYP2C9*3/*3* variant require approximately from 3.5 to 10-fold lower dose of warfarin to achieve therapeutic INR compared to the *CYP2C9*1/*1* variant [10].

The carriers of the *CYP2C9*2* and/or *CYP2C9*3* allele, who represent 10% and 6% of the European population, respectively, have an increased risk of bleeding complications, especially at the beginning of anticoagulation therapy and need a longer time to stabilize the VKAs dose [2, 11, 12]. The risk of bleeding complications during warfarin treatment has been increased by 90% for *CYP2C9*2* and by 80% for *CYP2C9*3* allele variants [2]. However, this data was derived mostly from studies performed in patients for whom the most frequent indication for warfarin treatment was AF (up to 60%), and then venous thromboembolism (VTE) (up to 40%). Patients after heart valve replacement have constituted less than 10% of all participants in the large published studies regarding the role of genetic polymorphisms in the optimization of VKA therapy [13–15].

Patients following mitral or aortic valve implantation have a relatively high risk of bleeding complications and exhibit a slightly different profile of bleeding and thromboembolic risk as compared to those with AF. It has been reported that total bleeding incidence is higher in patients after mitral valve replacement (32.3%) than in those after aortic valve replacement (21.8%), whereas the incidence of clinically relevant moderate or severe bleeding is not significantly different among the groups during 13 months of follow-up [16]. Similarly, Schapkaitz et al. have reported that there was no significant difference in overall bleeding incidence between the mechanical valve replacement subgroups and the control group over a 4-month follow-up [17]. However, patients with double (aortic and mitral) valve replacements had a higher proportion of combined bleeding and thromboembolic complications (30.61%) than patients with single aortic or mitral valve replacements (14.29% vs. 18.05%, respectively) and patients in the control group (12.87%) [17].

To the best of our knowledge, most of the studies concerning the genetic associations with quality of anticoagulation and adverse events have been performed on AF and VTE patients and the subjects after heart valve replacement have not been analyzed separately. Therefore, we sought to evaluate the relationship between *CYP2C9* polymorphisms and TTR in this subset of anticoagulated patients in whom INR was monitored using 2 different approaches, i.e. self-testing and standard care.

Aim of the Study

The aim of study was to investigate how allelic variants in *CYP2C9* affect the stability of anticoagulation with warfarin in patients within the first 3 months following elective heart valve replacement.

Material and Methods

Between March and October 2013, 18 consecutive carriers of *CYP2C9 *2* and/or **3* and 25 patients with wild type *CYP2C9 *1/*1* were enrolled. The inclusion criteria were: age of at least 18 years, elective aortic and/or mitral valve replacement and need to use warfarin for at least 3 months. The exclusion criteria were: acute infective endocarditis, renal failure (creatinine > 200 umol/L), acute

vascular incident within 3 months prior to enrollment, psychiatric diseases, alcoholism, the use of thienopyridines, oral corticosteroids or immunosuppressive agents. Subjects receiving drugs interfering with warfarin, including amiodarone, rifampicin, carbamazepine, antifungal azole agents, ritonavir and barbiturates, were ineligible.

Demographic and clinical characteristics of the patients were recorded prior to surgery. Arterial hypertension was diagnosed based on blood pressure > 140/90 mm Hg or preadmission of antihypertensive treatment. Smoking was defined as the daily use of 1 or more cigarettes. Patients receiving insulin or oral hypoglycemic drugs, or having at least 2 random fasting glucose levels of > 7 mmol/L were classified as having diabetes mellitus. The left ventricular ejection fraction was measured by Doppler echocardiography using the modified Bernoulli equation. All patients gave written informed consent and the study was approved by the University Bioethical Committee.

Carriers of mutations in *CYP2C9* were randomly assigned to 2 groups: (1) using coagulometers at home to measure INR (Alere INRatio®2 PT/ /INR Monitoring Systems, ALERE™, San Diego, CA, USA) and (2) monitoring INR values in a standard manner at the primary care physician's or local cardiologist's discretion. The former group was instructed to use the point-of care INR devices weekly to self-test at home. The self-testing group received INR testing instruction, including data interpretation and coping with typical issues of the measurements. Training was initiated as soon after valve replacement as the patient's clinical state allowed and was completed before discharge. The first INR measurement was performed by a physician and thereafter by the patients. If there were any doubts in the measurement procedure, the patient was excluded from the self-testing group. In some cases, the patient's family member was also trained. When INR was out of the therapeutic range, the patient was required to consult with the physician.

The latter group and patients with the wild type *CYP2C9*1/*1* genotype measured INRs in local outpatient clinics at least once every 3–4 weeks. The estimated warfarin dose was obtained from the online algorithm (http://www.warfarindosing.org) for each patient within 72 h after the surgery. The first warfarin dose was administered after removal of the epicardial electrode (5–10 days after surgery). Further doses of warfarin were modified based on the INR measurements. Within the first 2 weeks, measurements were performed every 2–3 days and then every 7–9 days, until the maintenance dose was established. To reliably calculate TTR, we defined a sufficient number of available INRs within the first 3 months since surgery study as 3.

The target INR range was 2.5–3.5 for mitral and 2.0–3.0 for aortic heart valve in accordance with the European Society of Cardiology (ESC) guidelines. The quality of warfarin dosing was assessed by the percentage of TTR, as described by Rosendaal et al. [5]. The last INRs were determined at a follow-up outpatient visit in the hospital laboratory after 3 months (90 ± 13 days) since surgery. At that visit we recorded adverse events, including death, minor (nose bleeds, subconjunctival hemorrhage, bruises and skin hematomas) and severe bleeds (hemorrhagic stroke, gastrointestinal bleeding) as well as thromboembolic events (valve thrombosis, transient ischemic attack, stroke, myocardial infarction, venous thromboembolism or other thrombotic episode), that were documented in medical records and/or self-reported.

DNA was isolated from whole blood collected in EDTA using the QIAamp® DNA Blood Mini Kit (QIAGEN, Valencia, CA) and stored at –80°C until analysis according to the manufacturer's protocol. Genotyping of *VKORC1* (c.-1639G > A; rs9923231), *CYP2C9*2* (p.Arg144Cys, c.430C > T; rs1799853) and *CYP2C9*3* (p.Ile359Leu, c.1075A > C; rs1057910) SNPs was determined by the allelic discrimination test using TaqMan Genotyping assays and the ABI PRISM 7900HT Fast Real-Time PCR System (Life Technologies Co., Carlsbad, CA, USA; assays IDs: C_30403261_20, C_25625805_10 and C_27104892_10, respectively). The genotype determination was confirmed by positive and negative controls.

Statistical Analysis

The normality of data distribution was tested using the Shapiro-Wilk test. Data was shown as mean ± standard deviation or median (interquartile range), as appropriate. Differences between 2 independent groups were compared with a Student's *t*-test or Mann-Whitney *U* test. Differences between the estimated and actual warfarin doses were assessed by Wilcoxon test. Qualitative data was compared by the Fisher exact test. Distribution of genotypes was evaluated by the χ^2 test. A p-value < 0.05 was considered statistically significant. Analysis was performed with STATISTICA v. 9.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Patient characteristics are presented in Table 1. The prevalence of the *CYP2C9 *2* and **3* allele (for a total of 43 patients) was: 0.17: CC-30

Table 1. Patient characteristics

	Non-carriers of *CYP2C9*2/3* (n = 25)	**Carriers of *CYP2C9*2/3* (n = 18)**	**P-value**	**Self-testing (n = 8)**	**Clinical-testing (n = 10)**	**P-value**
Age (year)	63.7 ± 9.5	65.2 ± 7.3	0.38	63.0 ± 2.4	66.9 ± 9.4	0.27
Male, n (%)	17 (68)	11 (61.1)	0.44	4 (50)	7 (70)	0.35
BMI (kg/m^2)	29.0 ± 4.2	28.3 ± 3.7	0.35	28.6 ± 4.4	28.1 ± 3.2	0.82
Comorbidities						
Arterial hypertension, n (%)	22 (88)	14 (77.8)	0.31	5 (62.5)	9 (90)	0.21
Hypercholesterolemia, n (%)	23 (92)	14 (77.8)	0.19	6 (75)	8 (80)	0.62
Diabetes, n (%)	7 (28)	3 (16.7)	0.31	1 (12.5)	2 (20)	0.59
CAD, n (%)	11 (44)	6 (33.3)	0.35	1 (12.5)	5 (50)	0.12
Atrial fibrillation, n (%)	9 (36)	7 (38.9)	0.55	3 (37.5)	4 (40)	0.65
LVEF (%)	55.0 (47.0–60.0)	59.5 (50.0–67.0)	0.30	52.0 (42.5–67.5)	62.0 (55.0–67.0)	0.45
Symptomatic HF, n (%)	0	2 (11.1)	0.17	1 (12.5)	1 (10)	0.71
Current smoking, n (%)	2 (8)	0	0.33	0	0	n.a.
Medications						
Beta-blockers, n (%)	23 (92)	15 (83.3)	0.34	5 (62.5)	10 (100)	0.07
Aspirin, n (%)	11 (44)	11 (61.1)	0.21	5 (62.5)	6 (60)	0.65
VKA, n (%)	9 (36)	6 (33.3)	0.56	2 (25)	4 (40)	0.44
Statin, n (%)	20 (80)	12 (66.7)	0.26	6 (75)	6 (60)	0.44
ACEI, n (%)	15 (60)	10 (55.6)	0.51	5 (62.5)	5 (50)	0.48
Surgery and its complications						
Aortic valve replacement, n (%)	20 (80)	14 (77.8)	0.58	5 (62.5)	9 (90)	0.21
Mitral valve replacement, n (%)	3 (12)	2 (11.1)	0.66	1 (12.5)	1 (10)	0.71
Both valves replacement, n (%)	2 (8)	2 (11.1)	0.56	2 (25)	0	0.18
Major thrombotic complications, n (%)	1 (4)	1 (5.5)	0.67	0	1 (10)	0.56
Minor bleeding complications, n (%)	5 (20)	4 (22.2)	0.58	2 (25)	2 (20)	0.62
Anticoagulant therapy						
TTR (%)	75.4 (58.1–83.6)	56.0 (38.6–74.9)	0.03	42.5 (36.0–55.7)	62.4 (54.5–79.6)	0.08
Above TTR (%)	4.5 (0.0–15.3)	13.8 (4.9–34.5)	0.05	10.2 (2.5–32.6)	16.0 (8.1–34.5)	0.63
Below TTR (%)	16.7 (8.2–32.1)	6.7 (0.0–58.2)	0.56	45.1 (2.0–59.5)	1.2 (0.0–27.1)	0.25
Number of INR measurements	7.9 ± 2.5	9.0 ± 2.9	0.2	10.1 ± 3.2	8.1 ± 2.3	0.14
Warfarin estimated dose (mg/week)	35.0 (28.0–42.0)	21.0 (21.0–35.0)	0.02	21.0 (21.0–31.5)	21.0 (21.0–35.0)	0.69

Table 1. Patient characteristics (cn.)

	Non-carriers of *CYP2C9*2/3* (n = 25)	**Carriers of *CYP2C9*2/3* (n = 18)**	**P-value**	**Self-testing (n = 8)**	**Clinical-testing (n = 10)**	**P-value**
Warfarin actual mean dose (mg/week)	46.3 ± 13.9	27.8 ± 13.2	< 0.001	30.1 ± 16.3	27.1 ± 11	0.78
Differences between the estimated and actual warfarin dose (mg/week)	9.0 (6.5–22.0) p = 0.001*	4.5 (3.0–10.5); p = 0.39*	0.03	8.8 (5.0–14.0); p = 0.32*	9.0 (4.0–21.0); p = 0.84*	0.17
Genotyping						
*CYP2C9*2*, n (%)	0	12 (66.7)	< 0.001	6 (75)	6 (60)	0.44
*CYP2C9*3*, n (%)	0	5 (27.8)	0.009	1 (12.5)	4 (40)	0.23
Both allelic variants of *CYP2C9*, n (%)	0	1 (5.5)	0.42	1 (12.5)	0	0.44
VKORC1 -1639G > A, n (%)	19 (76)	12 (66.7)	0.37	4 (50)	8 (80)	0.20

Data is given as mean ± SD, median (interquartile range) or number (percentage). ACEI – angiotensin-converting enzyme inhibitor; BMI –body mass index; CAD – coronary artery disease; *CYP2C9* – cytochrome P450 2C9; HF – heart failure; LVEF – left ventricular ejection fraction; TTR – time within therapeutic range; VKA – Vitamin-K antagonist; *VKORC1* – – vitamin K epoxide reductase complex subunit 1.
* – association between the estimated and actual warfarin doses within a group.

(69.8%), CT-11 (25.6%) and TT-2 (4.6%) and 0.07: AA-37 (86%), AC-6 (14%) and CC-0 (0%), respectively. The distribution of *CYP2C9 *2* and CYP2C9**3* genotypes was in accordance with the Hardy-Weinberg equilibrium (p = 0.54 and 0.24, respectively).

The patients with *CYP2C9 *2* and/or **3* allele and the control subjects with the *CYP2C9 *1/*1* genotype did not differ with regard to demographic data, clinical factors, the prevalence of *VKORC1* -1639G > A polymorphism and number of INRs measurements (Table 1).

In the group with *CYP2C9 *2* or **3* allele, there were 14 (77.8%) patients following aortic, 2 (11.1%) following mitral and 2 (11.1%) with both aortic and mitral valve replacement. The *CYP2C9**2 allelic variant was observed in 12 (66.7%) patients, while the *CYP2C9*3* allelic variant was found in 5 (27.8%) patients. There was one (5.5%) patient with the both variants. The *VKORC* -1639G > A polymorphism, i.e. the GA or AA genotypes, was detected in 12 (66.7%) patients with the *CYP2C9 *2* or **3* allele.

During the first 3 postoperative months, no death or hemorrhagic or ischemic strokes were noted in either group. Transient ischemic attack occurred in 1 (4%) patient with the *CYP2C9*1*1* variant and in 1 (5.5%) patient with a *CYP2C9*1*3* mutation. Gastrointestinal bleeding was observed in 2 (8%) patients without mutation in the *CYP2C9* gene. Subconjunctival hemorrhage was observed in 2 (11%) patients with *CYP2C9*1*3* and *CYP2C9*2*3* mutations. Nosebleed was recorded in 1 patient with the *CYP2C9*2* variant. Bruises and skin hematomas were reported by one (5.6%) patient with the *CYP2C9*2* mutation and in 3 (12%) patients with the *CYP2C9*1*1* genotype. Taken together, there were no differences between groups in the incidence of thromboembolic and hemorrhagic complications.

As expected, the carriers of *CYP2C9*2* and/or **3* variants had 40% lower both estimated and actual warfarin doses than the patients with the wild type *CYP2C9*1/*1* genotype. In contrast to the wild-type carriers, no differences between the estimated and actual warfarin doses in patients having *CYP2C9*2* and/or **3* variants were observed (Table 1).

The carriers of mutations in *CYP2C9* were characterized by lower TTR (56.0 [38.6–74.9] vs. 75.4 [58.1–83.6] %, p = 0.03) and spent a longer time in which the INR was above the therapeutic range (13.8 [4.9–34.5] vs. 4.5 [0–15.3] %, p = 0.047) than the wild type patients.

There were no significant differences between the self-testing (n = 8, 44%) and standard-care (n = 10, 56%) groups with regard to demographic and clinical characteristics (Table 1). There were also no differences in the frequency of the studied allelic variants. However, in the self-testing group, the *CYP2C9*3* variant was less frequent than in the standard-care group (1 carrier, 12.5% vs. 4 carriers,

40%, respectively), with one patient (12.5%) with both mutated *CYP2C9* allelic variants. No differences in the estimated and actual warfarin doses, TTR values and adverse events between the self-testing and standard-care subgroups were observed.

Discussion

The current study demonstrated that in patients following elective heart valve replacement the common allelic *CYP2C9*2* and/or **3* variants are associated with lower warfarin dosing and lower TTR in comparison to the *CYP2C9*1/*1* genotype evaluated within the first 3 months of anticoagulation. This finding is consistent with previous reports published mainly for patients with AF and/or VTE [13–15, 18]. Importantly, our study shows a similar impact of the two *CYP2C9* mutations on the quality of anticoagulation in heart valve recipients with several comorbidities, e.g. heart failure, which might be perceived as factors that contribute to large variations in TTR and INR values.

The TTR value of the most common patient group, i.e. subjects with the *CYP2C9*1/*1* genotype, was 75.4% and was even higher or similar to those reported previously, mostly for AF patients [13, 19, 20]. This highlights the good quality of warfarin therapy in Polish patients after valve implantation, which indicates a large improvement in TTR values in everyday practice nowadays, although compared to countries with the best quality of anticoagulation, e.g. Sweden, Poland, without anticoagulation clinics, still has an overall suboptimal quality of long-term VKA treatment.

In the current study, patients possessing mutated allelic variants of the *CYP2C9* gene had lower both estimated and actual warfarin doses than patients with the *CYP2C9*1/*1* genotype. This is in line with the previous reports concerning Turkish and Lithuanian patients after heart valve replacement where carriers of *CYP2C9*2* and/or *CYP2C9*3* genotypes required a lower maintenance dose of warfarin than patients with the *CYP2C9*1/*1* wild-type genotype [21, 22]. Furthermore, in our study, no differences between the estimated and actual warfarin doses and no significant bleeding complications in carriers of *CYP2C9*2* and/or **3* were observed. On the contrary, in the *CYP2C9*1*1* group, differences between the estimated and actual warfarin doses were observed. It may be speculated that in our study group the pharmacogenetics algorithm is less helpful for patients without mutations in *CYP2C9* requiring the intermediate warfarin dosing, as has been found previously [23]. The International Warfarin Pharmacogenetics Consortium has reported that a pharmacogenetics algorithm was more accurate for patients requiring lower (≤ 21 mg per week) or higher doses (≥ 49 mg per week) of warfarin than a clinical algorithm. For patients requiring ≤ 21 mg per week, the pharmacogenetic algorithm has given 50% of patients a well-predicted dose of warfarin, while the clinical algorithm has given only 33% [23]. In our study, we confirmed that the pharmacogenetic algorithm may be a useful for the estimation of appropriate anticoagulation with warfarin in patients who require the lower warfarin doses. However, in this group of patients, the strong influence of *CYP2C9* variants was demonstrated in a less stable anticoagulation reflected by shorter TTR and a longer period where the INR was above the therapeutic range. These results might suggest that careful warfarin dosing and more frequent INR testing in the group with *CYP2C9*2* or **3* variants are necessary.

INR testing can be done in outpatient clinics or by patients themselves at home. Self-testing of INRs has become more and more popular in Europe. In the current study, no differences were found in TTR or adverse event occurrence between the self-testing and standard-care groups. Similarly, previous studies in a large cohort of patients mostly with AF and after mechanical heart valve implantation have shown no differences in frequency of the first stroke, major bleeding complications and death between the self-testing and standard-care group [24]. However, better TTR has been achieved in the self-testing group than the standard care group [19, 24]. We did not find these associations probably due to the limited size of our study group. Similarly, bleeding episodes were not associated with the presence of the mutated variants of *CYP2C9*, probably due to the small number of patients. However, in a recently reported case-control study where patients after heart valve replacement constituted 20 and 10%, respectively, the *CYP2C9*2/*3* and *VKORC1* variants showed only a non-significant trend toward increased major bleeding risk in the initial 6 months of warfarin use [25]. It might be concluded that self-testing in everyday practice among mostly elderly patients is not superior to the standard care if the INR measurements are performed regularly, the patients are instructed on the risk and benefits from the therapy and modification of warfarin dosage is promptly recommended. Self-testing should be limited to anticoagulated patients who have objective obstacles to measure INR in outpatient clinics, e.g. logistical problems to reach them at least every 3–4 weeks.

Limitations

First, the major limitation of the current study is the small size of the patient groups. However, the impact of the 3 genetic polymorphisms evaluated

in the context of TTR was potent enough to be observed. Second, follow up in our study was restricted to the first three postoperative months, and a longer follow-up might provide additional data, including the postoperative long-term clinical outcome, especially bleedings associated with the various genotypes. Finally, the current observations cannot possibly be extrapolated on patients receiving other VKAs, in particular acenocoumarol, which is still the most commonly used VKA in Poland. There are some differences in the role of the polymorphisms studied in the stability of anticoagulation with acenocoumarol versus warfarin [9].

The authors concluded that *CYP2C9*2* and/or **3* variants increase the risk of unstable anticoagulation with warfarin in patients within the first 3 months following heart valve replacement. INR--self testing does not deteriorate the anticoagulation control in patients with *CYP2C9* variants in comparison to patients who monitor the INR in local outpatient clinics. Genotyping of *CYP2C9*2* and/or **3* variants might improve outcome in patients after heart valve replacement.

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References

[1] **Stein PD, Alpert JS, Bussey HI, Dalen JE, Turpie AG:** Antithrombotic therapy in patients with mechanical and biological prosthetic heart valves. Chest 2001, 1, 220–227.
[2] **Sanderson S, Emery J, Higgins J:** CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med 2005, 7, 97–104.
[3] **Kornej J, Potpara T, Lip GY:** Anticoagulation management in nonvalvular atrial fibrillation: current and future directions. Pol Arch Med Wewn 2013, 123, 623–634.
[4] **Furie B, Bouchard BA, Furie BC:** Vitamin K-dependent biosynthesis of c-carboxyglutamic acid. Blood 1999, 93, 1798–1808.
[5] **Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E:** A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost 1993, 69, 236–239.
[6] **Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G:** American College of Chest Physicians. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence--Based Clinical Practice Guidelines, 8th ed. Chest 2008, 133, 160–198.
[7] **Wadelius M, Chen LY, Eriksson N, Bumpstead S, Ghori J, Wadelius C, Bentley D, McGinnis R, Deloukas P:** Association of warfarin dose with genes involved in its action and metabolism. Hum Genet 2007, 121, 23–34.
[8] **Cavallari LH, Shin J, Perera MA:** Role of pharmacogenomics in the management of traditional and novel oral anticoagulants. Pharmacotherapy 2011, 31, 1192–1207.
[9] **Ufer M:** Comparative pharmacokinetics of vitamin K antagonists: warfarin, phenprocoumon and acenocoumarol. Clin Pharmacokinet 2005, 44, 1227–1246.
[10] **Johnson JA, Gong L, Whirl-Carrillo M, Gage BF, Scott SA, Stein CM, Anderson JL, Kimmel SE, Lee MT, Pirmohamed M, Wadelius M, Klein TE, Altman RB:** Clinical Pharmacogenetics Implementation Consortium. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther 2011, 90, 625–629.
[11] **Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE:** Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. JAMA 2002, 287, 1690–1698.
[12] **Aithal GP, Day CP, Kesteven PJ, Daly AK:** Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999, 353, 717–719.
[13] **Skov J, Bladbjerg EM, Leppin A, Jespersen J:** The influence of VKORC1 and CYP2C9 gene sequence variants on the stability of maintenance phase warfarin treatment. Thromb Res 2013, 131, 125–129.
[14] **Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, Holm L, McGinnis R, Rane A, Deloukas P:** The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009, 113, 784–792.
[15] **Jorgensen AL, Al-Zubiedi S, Zhang JE, Keniry A, Hanson A, Hughes DA, Eker DV, Stevens L, Hawkins K, Toh CH, Kamali F, Daly AK, Fitzmaurice D, Coffey A, Williamson PR, Park BK, Deloukas P, Pirmohamed M:** Genetic and environmental factors determining clinical outcomes and cost of warfarin therapy: a prospective study. Pharmacogenet Genomics 2009, 19, 800–812.
[16] **Hering D, Piper C, Bergemann R, Hillenbach C, Dahm M, Huth C, Horstkotte D:** Thromboembolic and bleeding complications following St. Jude Medical valve replacement: results of the German Experience with Low--Intensity Anticoagulation Study. Chest 2005, 127, 53–59.
[17] **Schapkaitz E, Jacobson BF, Becker P, Conway G:** Thrombo-embolic and bleeding complications in patients with mechanical valve replacements – a prospective observational study. S Afr Med J 2006, 8, 710–713.
[18] **Ferder NS, Eby CS, Deych E, Harris JK, Ridker PM, Milligan PE, Goldhaber SZ, King CR, Giri T, McLeod HL, Glynn RJ, Gage BF:** Ability of VKORC1 and CYP2C9 to predict therapeutic warfarin dose during the initial weeks of therapy. J Thromb Haemost 2010, 8, 95–100.

[19] Thompson JL, Burkhart HM, Daly RC, Dearani JA, Joyce LD, Suri RM, Schaff HV: Anticoagulation early after mechanical valve replacement: improved management with patient self-testing. J Thorac Cardiovasc Surg 2013, 146, 599–604.
[20] Singer DE, Hellkamp AS, Piccini JP, Mahaffey KW, Lokhnygina Y, Pan G, Halperin JL, Becker RC, Breithardt G, Hankey GJ, Hacke W, Nessel CC, Patel MR, Califf RM, Fox KA, ROCKET AF Investigators: Impact of global geographic region on time in therapeutic range on warfarin anticoagulant therapy: data from the ROCKET AF clinical trial. J Am Heart Assoc 2013, 2, e000067.
[21] Tatarūnas V, Lesauskaitė V, Veikutienė A, Jakuška P, Benetis R: The influence of CYP2C9 and VKORC1 gene polymorphisms on optimal warfarin doses after heart valve replacement. Medicina (Kaunas) 2011, 1, 25–30.
[22] Yildirim H, Tamer L, Sucu N, Atik U: The role of CYP2C9 gene polymorphisms on anticoagulant therapy after heart valve replacement. Med Princ Pract 2008, 6, 464–467.
[23] International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA: Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009, 360, 753–764.
[24] Matchar DB, Jacobson A, Dolor R, Edson R, Uyeda L, Phibbs CS, Vertrees JE, Shih MC, Holodniy M, Lavori P, THINRS Executive Committee and Site Investigators: Effect of home testing of international normalized ratio on clinical events. N Engl J Med 2010, 363, 1608–1620.
[25] Roth JA, Boudreau D, Fujii MM, Farin FM, Rettie AE, Thummel KE, Veenstra DL: Genetic risk factors for major bleeding in patients treated with warfarin in a community setting. Clin Pharmacol Ther 2014, 6, 636–643.

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